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Epidemiology of respiratory syncytial virus in a community birth cohort of infants in the first 2-years of life

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ABSTRACT

Respiratory syncytial virus (RSV) is the most common virus identified in children hospitalised with acute respiratory infections. However, less is known about RSV in community settings. This report describes RSV epidemiology in the community, including acute illness episodes, healthcare burden, and risk factors in Australian children during the first 2-years of life. A community-based, birth-cohort from Brisbane, Australia, followed children until their second birthday. Parents completed daily respiratory symptom and illness-burden diaries. Weekly parent-collected nasal swabs were analysed for RSV by real-time polymerase chain reaction assays. Serum RSV neutralising antibodies were assayed at age 3-years. Overall, 158 children provided 11,216 swabs, of which 104 were RSV positive (85 incident episodes). RSV incidence in the first 2-years of life was 0.46 (95%CI=0.37─0.58) episodes per child-year. Incidence increased with age, formal childcare attendance and was highest in autumn. Of 82 episodes linked with symptom data, 60 (73.2%) were symptomatic, 28 (34.1%) received community-based medical care, and 2 (2.4%) led to hospitalisation. Viral load was higher in symptomatic than asymptomatic infections. In 72 children, RSV-specific antibody seroprevalence was 94.4% at age 3-years.

Conclusion: RSV incidence increased after age 6-months with approximately three-quarters of infections symptomatic and most infections treated in the community.

Keywords: community birth cohort, respiratory syncytial virus, acute respiratory infection, infant, child

Word Count: 3070 words; **Abstract:** 199 words

INTRODUCTION

Respiratory syncytial virus (RSV) is the leading cause of hospitalisation for acute lower respiratory infections (ALRI) among young children globally [1; 2]. In 2015, respiratory syncytial virus (RSV) caused an estimated 59,600 deaths and 3.2 million hospitalisations for ALRIs worldwide in children aged <5-years [2]. In Australia, 56,639 children aged <24-months were hospitalised between 2006 and 2015, with 36% aged <3-months [3]. The World Health Organization has declared developing RSV vaccines a priority [4]. However, despite RSV vaccines undergoing development and clinical trials [5], important knowledge gaps in RSV epidemiology remain to guide public health and vaccine policy [6]. Studies describing RSV infections in children are mostly hospital-based and thus likely restricted to those with severe disease [7-11]. Although by 2-years of age most children are believed to have had RSV [12], community-level data remain limited, especially describing the associated healthcare burden [13].

An Australian prospective community-based birth cohort, the Observational Research in Childhood Infectious Diseases (ORChID) study, reported the median age at first RSV detection as 19.4-months, with 58.4% of children having RSV infection by age 24-months [14]. RSV was strongly associated with ALRI, such that when children had detectable RSV, 68% of ARLIs were attributable to the RSV episode [15]. The aim of this study was to use ORChID data to further describe the epidemiology and health-service use related to RSV detections, and to investigate associations with co-detected pathogens, and with child and family characteristics, in the first 2-years of life.

METHODS

Study population

The ORChID study (clinicaltrials.gov: NCT01304914), which has been described previously [16], enrolled healthy term newborn babies in Brisbane, Australia. Enrolment was progressive between September 2010 and October 2012, which allowed for seasonal and year-to-year variation in respiratory virus infections. The children were followed until their parents stopped mailing in diaries and nasal swabs, or when the children reached their second birthday (see Supplementary methods for further details). The Children's Health Queensland (HREC/10/QRCH/16), Royal Brisbane and Women's Hospital (HREC/10/QRBW/125) and the University of Queensland (2010000820) human research ethics committees approved the ORChID study. Griffith University (2020/197) approved this secondary data analysis.

Sociodemographic characteristics

At enrolment, parents provided sociodemographic and health characteristics, including pregnancy and birth details. They were subsequently interviewed by telephone every 3-months to update information on feeding and childcare arrangements. Childcare was categorised as formal (regulated care outside the child's home) and informal (non-regulated care by family or friends). A child was defined as being exclusively breastfed at age 4-months if by this age they had been only breastfed without any milk formula or solid food [16].

Study procedures

Parents completed a daily tick-box diary of pre-defined respiratory symptoms, which research staff had trained them to recognise [17] (Supplementary Figure 1). An acute respiratory infection (ARI) was defined as an upper respiratory infection (URI) when nasal congestion or nasal discharge, dry cough, or doctor-diagnosed acute otitis media (AOM) was recorded, and as an ALRI when any of moist cough, rattle-like breathing, shortness of breath, wheeze, or

doctor-diagnosed pneumonia was entered in the symptom diary. Fever was recorded and could occur with either URI or ALRI episodes. ARIs were sub-categorised hierarchically into either an ALRI or URI respectively. Three or more symptom-free days demarcated new ARI episodes. Parents kept illness-burden diaries to capture healthcare-seeking behaviour, including primary care, hospital visits and antibiotic prescriptions, when ARI symptomatology met a defined threshold (all ALRI, AOM, and URI with dry cough plus nasal symptoms). In order to minimise parent inconvenience, we did not ask for illness-burden diary entries for either isolated nasal symptoms or dry cough, and assumed parents did not seek healthcare under these circumstances. Completed diaries were returned to the research team each month by mail. Three-monthly telephone calls encouraged timely swab and diary returns to minimise attrition.

Parents were taught to collect anterior nasal swabs from their child at birth and continued taking these swabs weekly thereafter until the child's second birthday. Swabs were collected regardless of symptoms and surface-mailed to the study laboratory, where they were received a median 3-days [interquartile range (IQR) 2─4] after collection and stored at -80^{0}C. The median interval between swabs was 7-days [IQR 7─12].

At completion of the intensive data collection phase of the ORChID study, emergency department and hospital records of participants were reviewed by one of the authors (KG). In addition, parents and children were invited to participate in an extension of ORChID, the Early Life Lung Function (ELLF) study, where children were seen annually from their third to seventh birthdays [18].

Respiratory pathogen detection

Swabs were batch-tested for 17 respiratory viruses, including RSV-A and RSV-B, and eight bacterial pathogens by previously validated real-time polymerase chain reaction (PCR) assays [16; 19]. All respiratory virus and bacterial detections with cycle threshold (Ct) values <40 were considered positive. Ct values from real-time PCR are inversely proportional to the amplified nucleic acid in the sample and were used as semiquantitative markers of viral load. A 3.3 cycle difference represents approximately a 10-fold difference in nucleic acid load [20]. Specimen quality was assessed by testing for a marker of human genomic DNA, endogenous retrovirus-3 (ERV-3) [21]. Swabs with either undetectable ERV-3 or ERV-3 Ct values >38 were deemed to be of poorer quality and removed from incidence rate calculations.

A new RSV episode was defined as detecting either an RSV virus subtype for the first time or, if the same RSV subtype had been detected previously, at least 30-days after the last positive swab for that subtype. An RSV episode was classified as symptomatic if symptoms were first detected in the week prior to the first virus detection for that episode, or if symptoms were present in the week after the first virus detection.

RSV antibody assays

Serum anti-RSV-neutralising antibody levels were measured in those participating in the ORChID follow-up study, the ELLF study, where blood samples were collected at age 3-years. Details of the antibody assays are provided in the Supplementary methods.

Analysis

The associations between RSV detections, ARIs and healthcare use were tabulated. The association between RSV swabs and other respiratory virus and bacterial detections was

investigated using log-binomial regression. Incidence rates of new RSV episodes, and associations between pre-defined risk factors and incident RSV episodes, were calculated using mixed-effects Poisson regression models, with child included as a random effect and models offset by the natural logarithm of child-years at risk. Each swab represented 7-days of study time. All multivariable models adjusted for age, season of detection, older child in the household at birth and childcare attendance. Associations between both presence of symptoms and episode duration with viral loads were assessed using linear regression. Data were analysed using Stata v13 (StataCorp, College Station, TX, USA).

RESULTS

One-hundred and fifty-eight children returned 11,126 swabs, and 154 children provided 87,547 symptom diary-days of observation (Figure 1). This included 10,811 swabs matched to 82,036 diary-days from 154 children and 8,101 higher-quality swabs from 157 children (equivalent to 155.4 child-years). Most were born between 39─41 weeks gestational age, were first-born children, and had a mother with a university degree. The 85 children who continued into the ELLF study had similar characteristics to the original ORChID cohort (Table 1).

RSV was detected in 104 swabs, of which 85 were incident episodes from 71 children. RSV-A was more prevalent (78 detections, 62 incident episodes from 56 children) than RSV-B (27 detections, 24 incident episodes from 24 children). This included nine children, who at different times had separate RSV-A and RSV-B episodes, and one child with a single RSV-A and RSV-B co-detection episode. Overall, 64 (78.0%) incident-episodes had a shedding duration of 1-week with a maximum duration of 5-weeks (Supplementary Table 1). Peak Ct values were significantly higher (lower load) for asymptomatic episodes (mean difference=3.5, 95% confidence interval [CI]=1.7─5.3; Table 2). A sensitivity analysis was

conducted to investigate whether this finding was influenced by incident episodes without swabs in the preceding week, but results were unchanged (Supplementary Table 2). Shedding duration was not associated with either symptoms (Supplementary Table 1) or peak viral loads (Supplementary Table 3).

Six children had more than one RSV infection (all RSV-A) a median of 6.4 (IQR=5.3─8.9) months apart (Supplementary Table 4). When RSV was detected in higher quality swabs, other respiratory viruses were also present on 26 (28.9%) occasions, while there were 65 (72.2%) instances where potential respiratory bacterial pathogens colonising the anterior nasal space were co-detected with RSV (Supplementary Table 5). Human rhinovirus had a negative association with RSV (adjusted relative risk [aRR]=0.38, 95%CI=0.22─0.68), while human polyomaviruses WU/KI had a positive association (aRR=2.37, 95%CI=1.09─5.11) (Supplementary Table 6).

The overall RSV incidence rate in the first 2-years of life was 0.46 (95%CI=0.37─0.58) episodes per child-year, with an incidence of 0.35 (95%CI=0.24─0.50) and 0.60 (95%CI=0.44─0.81) episodes per child-year in the first and second-years respectively (Table 3). The overall incidence rate for symptomatic RSV infections was 0.33 (95%CI=0.25─0.44) episodes per child-year. RSV demonstrated seasonality (Supplementary Figure 2). More incident RSV episodes occurred during autumn (March-to-May), than in summer (December-to-February) months (adjusted incidence rate ratio [aIRR]=3.22, 95%CI=1.60─6.48). Similarly, increased incidence was associated with formal childcare attendance, compared to no attendance (aIRR=2.00, 95% CI=1.08─3.71) (Supplementary Table 7). There was no significant association between RSV incidence and sex, season of birth,

type of delivery, exclusive breastfeeding duration, family history of atopy, tobacco smoke exposure, older children in household or maternal education.

There were 82 (96.5%) episodes linked to symptom diaries, of which 60 (73.2%) were associated with an ARI. Both RSV-subtypes had a similar percentage of episodes associated with ALRI (Supplementary Table 1). Co-detection of RSV with other respiratory viruses or potential respiratory bacterial pathogens was not associated with increased risk of respiratory symptoms (Supplementary Table 5). Only two ARI episodes (3.3%; both RSV-A) resulted in hospitalisation. Both children were diagnosed with acute bronchiolitis, one was 5.7-months of age and the other, aged 13.7-months, also had bilateral AOM treated with ceftriaxone. Of the 60 symptomatic episodes, 53 (88.3%) were managed solely within the community, including 26 (43.3%) who had no healthcare contact (Table 4). Of 19 children prescribed antibiotics, one (5.3%) had AOM. There were no children with doctor-diagnosed pneumonia.

At age 3-years, 68/72 (94.4%) ELLF children who provided blood were seropositive for RSV neutralising antibodies. One of the four seronegative children had a single RSV positive swab at age 15-months, which was associated with the beginning of a 22-day URI.

DISCUSSION

Healthy children from the ORChID cohort averaged 0.46 RSV infections (0.33 symptomatic) per child-year during the first 2-years of life. RSV was detected more frequently during the second than first-year of life, while independent risk factors for RSV were the seasonal autumn peak and formal childcare attendance. Overall, 73% of RSV episodes were symptomatic and,

of these, 97% were managed at home or as outpatients. There was almost a 10-fold difference in viral loads between children with symptoms and those with asymptomatic RSV infections.

The incidence-rate observed in ORChID children was lower than the seminal Houston Family Study conducted in 1976, which reported 0.68 and 0.82 RSV episodes per child-year for the first and second-years of life, respectively [12]. However, our rate agrees with more recent community-based birth-cohort studies. These include a Kenyan study conducted between 2002 and 2005 over three consecutive RSV seasonal epidemics (0.50 cases per child-year over the first 30-months of life) [22] and two others reporting the incidence of RSV-associated ARIs from birth until age 2-years, one from Finland in 2008─2010 (0.37 cases per child-year) [23] and another from Nicaragua in 2011─2016 (0.25 cases per child-year) [24]. The Kenyan study was similar in design to the Houston study and investigated the incidence of RSV infection in the community by following the children with home visits from birth until they had experienced three consecutive RSV epidemic seasons [22]. Rapid antigen detection testing during ARI episodes and 3-monthly blood samples for serology were used to detect RSV infections [22]. The Finnish [23] and Nicaraguan studies [24] only collected upper airway samples during ARIs and in the Finnish cohort blood was obtained for serology at 13, 24 and 36-months [23]. While the Houston study reported RSV-specific antibody seroprevalence of 97% at 13─24 months, similar to our own findings [14], the Finnish cohort study reported RSV seroprevalence of 37%, 68% and 86% at 1, 2 and 3-years of age respectively [12;25;26]. In addition, the Kenyan study reported 83% at 18─24 months and 100% after 3-years of age [25;26].

Primary RSV infections in the first 2-years of life are commonly believed to be symptomatic and more severe than at other times, requiring some kind of healthcare contact [12]. Consistent

with international and Australian-based population-based studies [11;27;28], two children in ORChID (~3% of episodes) required hospitalisation. While 57% of symptomatic RSV-ARI episodes resulted in healthcare contact, 27% of all RSV infections were asymptomatic. The seroprevalence results from the Finnish birth cohort also suggested subclinical RSV infections in this age group [25;26]. In contrast, asymptomatic episodes were lower in a Kenyan household study, which also collected weekly nasopharyngeal swabs and reported that in those aged <1-year and 1─4 years, 9.1% and 17.3% of episodes respectively were asymptomatic during an RSV season [29]. The median household size in the Kenyan study was eight members, which is more than twice that of the ORChID cohort and may help explain the different asymptomatic rates as household crowding is a recognised risk factor for RSV-ALRI [30].

These results have implications for public health policy for maternal vaccination and the introduction and timing of RSV vaccines in infancy. The highest rates of hospitalisation for RSV are in the first 3-months of life [3] and a recent large multi-national placebo-controlled randomised trial found maternal vaccination reduced hospitalisations from severe RSV-ALRI in this vulnerable age group (vaccine efficacy 44.4%; 95%CI=19.6─61.5) [31]. However, due to the decay in maternal antibodies [25], maternal vaccination is unlikely to have a significant impact on infants aged >3-months, who collectively comprise most hospitalised cases [3;32] and the far greater numbers with RSV-ARI managed in the community [13]. Previous population-based studies, including ORChID, have shown RSV-ALRI in otherwise healthy infants remains an important cause of morbidity between 3─12 months of age [14;22;24;33-35]. An alternative strategy is administering a single dose of a long-acting RSV-neutralising monoclonal antibody to infants entering the RSV season [36]. In the future,

approved paediatric vaccines for older infants could also offer additional public health benefits, including protecting older children who frequently introduce RSV into households [25;29;37].

The data on viral load and clinical severity in infants with RSV infections conflicts with studies showing either no difference [38], milder disease [39;40] or greater severity [41;42] with increased viral loads. These studies were predominantly hospital-based and often cross-sectional, which could help explain the observed differences between studies. In contrast, there are limited data from RSV infections managed within the community. A birth cohort study from the Netherlands collected nasopharyngeal samples from participants at the time of their first ARI [43]. In this study, the 30 infants with an RSV-associated ARI had a negative correlation between their RSV Ct values (ie. increased viral load) and clinical severity score ($r= -0.52$; $p<0.003$). A prospective study of ARI in Finnish children managed as outpatients found higher RSV loads (Ct <27) were associated with longer median symptom duration (10.0 vs 8.0-days; $p=0.02$), but as with our own study this association was not seen in children aged <2-years [44]. Additional support for the role of viral loads comes from adult volunteer challenge studies where a close temporal relationship between onset, peak, and clearance of RSV loads and the onset, peak, and resolution of symptoms has been observed [45]. Our study extends these observations by demonstrating a significantly higher viral load in those experiencing symptoms of an RSV-ARI compared with infants and young children with an asymptomatic infection. Taken together, these results support developing anti-viral agents to decrease viral loads and in so doing they may alter the course of an RSV illness.

Strengths and limitations Strengths of this analysis are the use of data from the ORChID study, an unselected healthy community-based birth cohort with high-density sampling spanning 4-years, which allowed us to address annual variations in RSV activity. The high-density specimen collection enabled the detection of both symptomatic and asymptomatic RSV

infections. Weekly swabs that can cover or bracket an illness episode increase the chances of virus detection rather than relying upon a single swab at the time of symptoms as other studies have done. Limitations include that ORChID participants were healthy and mostly first-born children from urban and more advantaged families, which may lead to an underestimate of RSV incidence, as children born preterm, and those from disadvantaged minority populations, overcrowded households and children exposed to tobacco smoke are at higher risk of RSV disease [30;46;47]. However, the relative effects we report are likely to be generalisable to most infants and young children residing in developed societies. Secondly, despite sensitive PCR assays, suboptimal swabbing techniques may have missed virus detections. Lower-quality swabs were therefore excluded from all incidence calculations to reduce the probability of including false-negative results and the consequent underestimation of incidence rates. A study from hospitalised children in Laos reported excellent agreement between nasal and nasopharyngeal swabs for RSV detection by real-time PCR [48], and we have shown recently that parent-collected nasal swabs have similar virus detection rates to those obtained by health personnel when employing PCR assays [49]. Thirdly, Ct values from real-time PCR assays are a proxy measure of viral load and can be influenced by individual assay performance characteristics. Nevertheless, large differences in Ct values are likely to be real. Finally, the diary symptoms were not validated by healthcare professionals other than for AOM and pneumonia. Although this means not all ARI episodes were confirmed by a healthcare professional, it is the only practical method of data collection in a longitudinal community cohort study of this type, and a similar diary format has previously been used successfully [50].

Conclusions

In contrast with hospital-based studies, RSV incidence in the community increases after age 3-months, with higher rates in the second-year of life, and by 3-years most children have been

infected. Although almost three-quarters of primary infections are symptomatic, and of these 57% seek healthcare, most children are managed within the community. While the current focus is on preventing RSV-ALRIs in the first months of life, there is also a considerable community burden imposed by RSV-ARIs in infancy and early childhood, and vaccines and other preventive measures should also be developed to protect this age group.

Declarations

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Conflicts of interest: No author reported a potential conflict of interest.

Ethics approval: The Children's Health Queensland (HREC/10/QRCH/16), the Royal Brisbane and Women's Hospital (HREC/10/QRBW/125) and The University of Queensland (2010000820) Human Research Ethics Committees approved the study.

Consent to participate: Individual mothers consented to participate antenatally for their children's participation. Mothers were re-consented at the start of ELLF study.

Consent for publication: Individual mothers consented for publication antenatally for their children's participation. Mothers were re-consented at the start of ELLF study.

Availability of data and material: De-identified participant data, the data dictionary, and related documents (eg, case report forms) will be made available on written request to the senior author. Requests must be accompanied by a formal protocol for the use of the data and approval from the relevant Human Research Ethics Committees. A written and signed data access agreement will be required**.**

Code availability: Contact corresponding author.

Author Contribution: MT had full access to all of the data in the study and had the final responsibility for the decision to submit for publication. Conception or design of the work: MT, KG, PS, SBL, KC, DW, RSW. Analysis and interpretation of data: MT, KG, PS, SBL, KC, DW, RSW. Initial drafting of the manuscript: MT. Critical revision of the manuscript for important intellectual content: KG, PS, SBL, KC, DW, RSW. Final approval of the version to be published: MT, KG, PS, SBL, KC, DW, RSW.

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Figure 1. Flow chart of nasal swabs and symptom diaries from children in the Observational Research in Childhood Infectious Diseases study. RSV = respiratory syncytial virus.

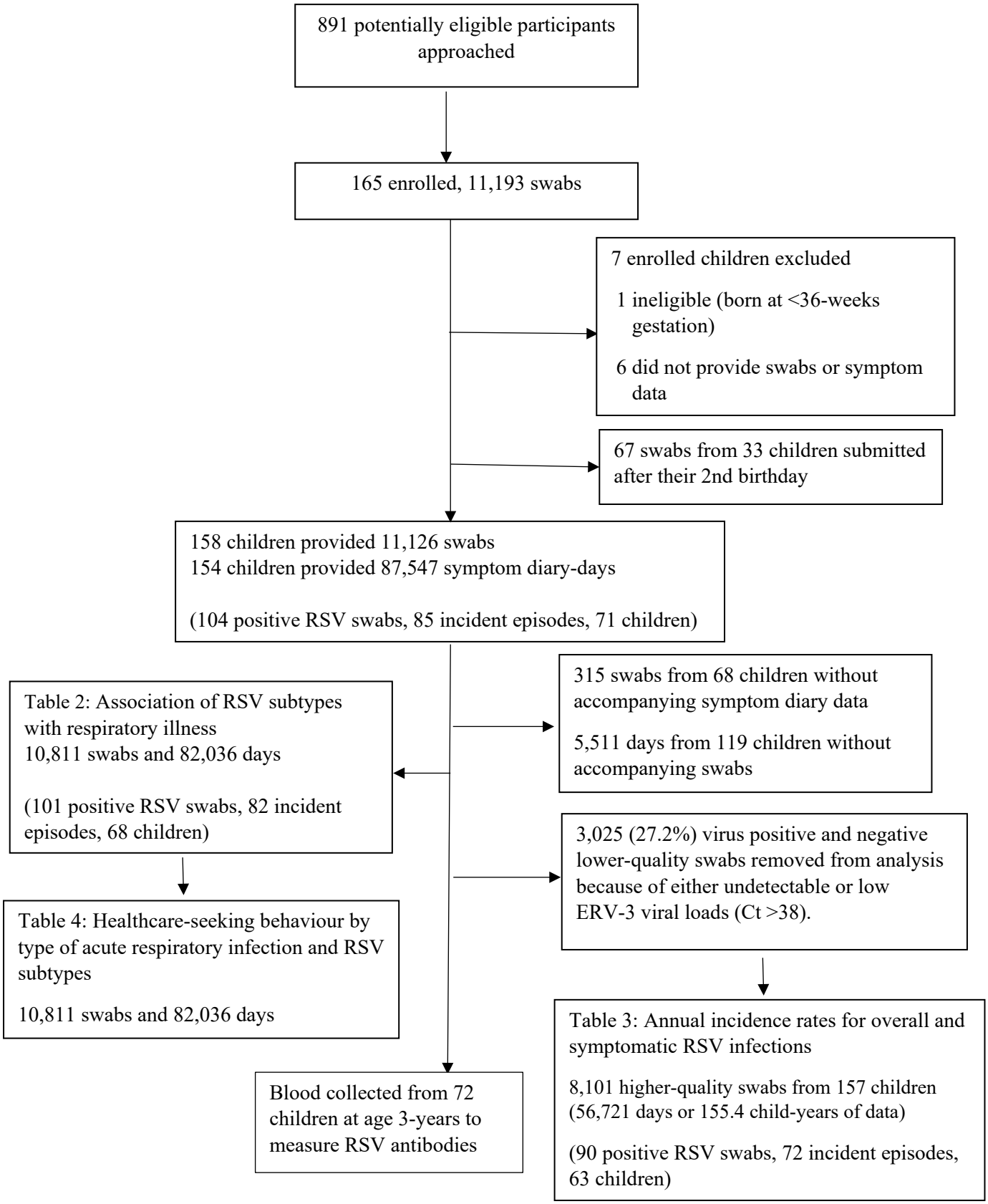

Table 1: Characteristics of the Observational Research in Childhood Infectious Diseases cohort and its extension, the Early Life Lung Function cohort

	ORChID (N=158)	**ELLF (N=85)**
	N (%)	N (%)
Gender (Male)	75 (47.5)	40 (47.1)
Season of birth		
Summer (December-February)	42 (26.6)	24 (28.2)
Autumn (March-May)	30 (19.0)	16 (18.8)
Winter (June-August)	43 (27.2)	22 (25.9)
Spring (September-November)	43 (27.2)	23 (27.1)
Vaginal delivery	107 (67.7)	56 (65.9)
Gestational age at birth*		
36-38 weeks	36 (22.8)	15 (17.7)
39-41 weeks	122 (77.2)	70 (82.4)
Family history		
Either parent has asthma/eczema	80 (50.6)	44 (51.8)
Household smoke exposure at birth	(n=156)	(n=85)
Yes	19 (12.2)	11 (12.9)
Older child(ren) in house at birth	55 (34.8)	30 (35.3)
Maternal education status	(n=157)	(n=85)
University degree	99 (63.1)	55 (64.7)
Diploma/certificate	38 (24.2)	21 (24.7)
Primary/secondary school	20 (12.7)	9 (10.6)
Mode of feeding	(n=153)	(n=85)
Exclusive BF until at least age 4-months	87 (56.5)	49 (57.7)
Childcare attendance at 6-months‡	(n=133)	(n=84)
No childcare	102 (76.8)	61 (72.6)
Informal childcare only	15 (10.9)	13 (15.5)
Formal childcare	17 (12.3)	10 (11.9)
Childcare attendance at 12-months‡	(n=116)	(n=78)
No childcare	44 (37.9)	25 (32.1)
Informal childcare only	21 (18.1)	21 (26.9)
Formal childcare	51 (44.0)	32 (41.0)
Childcare attendance at 18-months‡	(n=108)	(n=77)
No childcare	16 (14.8)	10 (12.9)
Informal childcare only	23 (21.3)	20 (25.9)
Formal childcare	69 (63.9)	47 (61.0)
Childcare attendance at 24-months‡	(n=103)	(n=71)
No childcare	17 (16.5)	9 (12.3)
Informal childcare only	18 (17.5)	15 (20.6)
Formal childcare	68 (66.0)	49 (67.1)

Abbreviation: BF: breastfeeding; ELLF: Early Life Lung Function; ORChID: Observational Research in Childhood Infectious Disease.

*Two participants were born between 36.0 and 36.6-weeks gestation.

‡Formal childcare was defined as outside homecare from a regulated childcare service, while informal care comprised non-regulated care by relatives, friends or neighbours.

Table 2: Association between peak RSV virus loads (inverse cycle threshold value) and presence of symptoms for each episode in children in the Observational Research in Childhood Infectious Diseases cohort (n=82 episodes from 154 children, 10,811 swabs, 82,036 person-days of observation).

	Asymptomatic Mean (SD)	Symptomatic Mean (SD) †	Mean Difference (95%CI)‡
RSV combined	33.2 (3.9) [n=22]	29.7 (3.6) [n=60]	3.5 (1.7, 5.3)
RSV-A	33.2 (3.9) [n=18]	29.4 (3.5) [n=41]	3.9 (1.8, 5.9)
RSV-B	33.1 (4.3) [n=4]	30.5 (3.7) [n=20]	2.6 (-1.7, 6.9)

Abbreviations: CI: confidence interval; RSV: respiratory syncytial virus; SD: standard deviation.

*104 RSV-positive swabs (85 episodes) in total, of which 101 RSV-positive swabs (82 episodes) were linked to symptom diaries.

†1 swab was positive for both RSV-A and RSV-B and was a symptomatic case.

‡ Linear regression was used with symptomatic episodes as the referent.

Table 3: Annual incidence rates for overall and symptomatic RSV infections in children in the Observational Research in Childhood Infectious Diseases cohort (n=157 children, 8,101 higher-quality swabs).

	IR (95%CI) First 2-years	IR (95%CI) Months 1-12	IR (95%CI) Months 13-24
RSV			
Overall	0.46 (0.37-0.58)	0.35 (0.24-0.50)	0.60 (0.44-0.81)
Symptomatic	0.33 (0.25-0.44)	0.27 (0.18-0.40)	0.41 (0.29-0.60)
RSV-A			
Overall	0.35 (0.27-0.45)	0.28 (0.19-0.42)	0.43 (0.30-0.61)
Symptomatic	0.24 (0.17-0.33)	0.20 (0.12-0.32)	0.29 (0.18-0.44)
RSV-B			
Overall	0.12 (0.08-0.19)	0.08 (0.04-0.17)	0.17 (0.10-0.30)
Symptomatic	0.10 (0.06-0.17)	0.08 (0.04-0.17)	0.13 (0.07-0.25)

Abbreviations: CI: confidence interval; IR: incidence rate per child-year; RSV: respiratory syncytial virus.

Table 4: Healthcare-seeking behavior by acute respiratory infection category and RSV subtype (10,811 swabs, 82,036 person-days of observation).

RSV subtype associated ARI	Symptomatic episodes*	Any healthcare contact (n, %)	Primary care visits† (n, %)	Other healthcare professional (n, %)	Hospital		Antibiotics (n, %)
					ED presentation only† (n, %)	Admission† (n, %)	
RSV combined‡							
ARI	60	34 (56.7)	33 (55.0)	2 (3.3)	5 (8.3)	2 (3.3)^	19# (31.7)
URI	26	10 (38.5)	10 (38.5)	0 (0.0)	1 (3.8)	0 (0.0)	6 (23.1)
ALRI	34	24 (70.6)	23 (67.6)	2 (5.9)	4 (11.8)	2 (5.9)	13 (38.2)
RSV-A							
ARI	41	22 (53.7)	22 (53.7)	2 (4.9)	4 (9.8)	2 (4.9)	15 (36.6)
URI	16	5 (31.3)	5 (31.3)	0 (0.0)	1 (6.3)	0 (0.0)	4 (25.0)
ALRI	25	17 (68.0)	17 (68.0)	2 (8.0)	3 (12.0)	2 (8.0)	11 (44.0)
RSV-B							
ARI	20	12 (60.0)	11 (55.0)	0 (0.0)	1 (5.0)	0 (0.0)	4 (20.0)
URI	11	5 (45.5)	5 (45.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)
ALRI	9	7 (77.8)	6 (66.7)	0 (0.0)	1 (11.1)	0 (0.0)	2 (22.2)

Abbreviations: ALRI: acute lower respiratory infection; AOM: acute otitis media; ARI: acute respiratory infection; ED: emergency department; RSV: respiratory syncytial virus; URI: upper respiratory infection.

*Illness-burden diary was completed when ARI symptomatology met a defined threshold (all ALRI, AOM, and URI with dry cough plus nasal symptoms). All 59 episodes fulfilling these criteria had an illness-burden diary submitted. One RSV episode did not meet threshold for burden diary completion (reported symptoms were runny nose only). Medical visits and antibiotic information were derived from the illness-burden diary.

†Medical visits were not mutually exclusive categories as a child may have more than one medical encounter in different settings during a single ARI episode.

‡Includes a symptomatic child who had both RSV-A and B co-detected in their nasal swab.

^ The ages of the two children hospitalised with RSV were 5.7 and 13.7-months.

Of the 19 children who were prescribed antibiotics, 1 (5.2%) had AOM. There were no children with doctor-diagnosed pneumonia.

SUPPLEMENTARY METHODS

Epidemiology of respiratory syncytial virus in a community birth cohort of infants in the first 2-years of life

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Abbreviations in the text:

Ct	Cycle threshold
ELLF	Early Life Lung Function
ERV-3	Endogenous retrovirus-3
GFP	green fluorescent protein
ORChID	Observational Research in Childhood Infectious Diseases
rgRSV	recombinant green fluorescent protein expressing respiratory syncytial virus
RSV	Respiratory syncytial virus

Eligibility criteria

The prospective Observational Research in Childhood Infectious Diseases (ORChID) birth cohort study of unselected healthy term infants was conducted in subtropical Brisbane, Australia (latitude 27° South, average monthly maximum temperature range 22-30°C, maximum rainfall in December-February, population 2.2 million). Recruitment was from antenatal clinics at one of two metropolitan hospitals (one private and one government-funded) over 2-years, which enabled infants born between September 2010 and October 2012 to be enrolled evenly throughout the study period and to be followed until their second birthday. This progressive enrolment allowed for seasonal and year-to-year variation in respiratory virus infections, including respiratory syncytial virus (RSV) [1]. Healthy infants born at term (36-42 weeks) without congenital abnormalities or underlying chronic disorders were included in the study. Exclusion criteria for enrolment and ongoing participation included gestational age <36-weeks at birth, major congenital abnormalities, chronic heart, respiratory (excluding asthma), gastrointestinal, neurological or immunological disorders, parents unable to converse in English, living outside the Brisbane metropolitan region or planning to move from the area within the next 2-years. Parents were interviewed by telephone every 3-months to update information on feeding, immunisation, childcare arrangements, cigarette smoking and any

changes to household numbers. In addition, the telephone calls served to encourage timely swab and diary returns in an attempt to minimise attrition. Children exited the study when we stopped receiving diaries and swabs, or when they had their second birthday. In order to minimise parent inconvenience, we did not ask for the illness-burden diary entries for isolated nasal symptoms or an isolated dry cough. We assumed that in these circumstances, parents would not seek healthcare advice, especially as this would involve a financial and time cost for most parents. Detailed methods of recruitment, study design, and data collection are described further in the published study protocol [1].

Swab collection

Swabs were collected weekly by parents from both anterior nares using a plastic-shaft, rayon-budded swab and inserted in a transport tube with a foam pad reservoir soaked with viral transport medium (Virocult MW950, Medical Wire & Equipment, Wiltshire, England). These were then surface mailed into the laboratory at ambient temperature where they were processed and stored at -80^0C.

Quality control

Specimen quality was assessed by testing for a marker of human genomic DNA, endogenous retrovirus-3 (ERV-3) [2]. Previously, we found that in specimens with an ERV-3 cycle threshold (Ct) value >38, respiratory virus detection declined significantly (odds ratio 0.35; 95% confidence interval 0.27, 0.44) compared to those with lower Ct values (higher ERV-3 load) [3]. Thus, swabs that were negative for ERV-3 or had an ERV-3 Ct value >38 were deemed to be of poorer quality and they, and their associated observation days, were removed from incidence rate calculations.

Antibody assays

ORChID parents and children were invited to participate in an extension of the ORChID study, the Early Life Lung Function (ELLF) study [4]. The children in ELLF had similar characteristics to the original ORChID cohort (Supplementary Table 1) and made annual visits to the research centre between 3 and 7-years of age where they underwent a series of assessments. At age 3-years this included a venous blood sample, which was centrifuged and stored at -80^{0}C until further analysed for serum RSV neutralising antibodies.

Virus neutralisation was assessed against recombinant green fluorescent protein (GFP)-expressing RSV (rgRSV) derived from D53, strain A2 that was provided by Prof Mark Peeples [5]. Vero cells were seeded into 96 well, optical bottom/black wall cell culture plates (Thermo Fisher Scientific) at a density of 4x10^4 cells per well in 100µl of Opti-MEM (Gibco) with 3% fetal calf serum and incubated overnight at 37°C, 5% CO_2. The following day, human plasma samples were titrated by 4-fold dilutions in Opti-MEM within a round bottom 96 well plate. Plasma dilutions were then mixed with 2 x 10^3 plaque-forming units/mL of rgRSV and incubated for 1-hour at room temperature before addition to Vero cells. Following 6-days incubation at 37°C, 5% CO_2, GFP fluorescence intensity was measured on a CLARIOstar Microplate Reader (BMG LABTECH, Melbourne Australia). Fluorescence was graphed against plasma dilution and a three-parameter dose response curve was fitted by nonlinear regression using GraphPad Prism 7.

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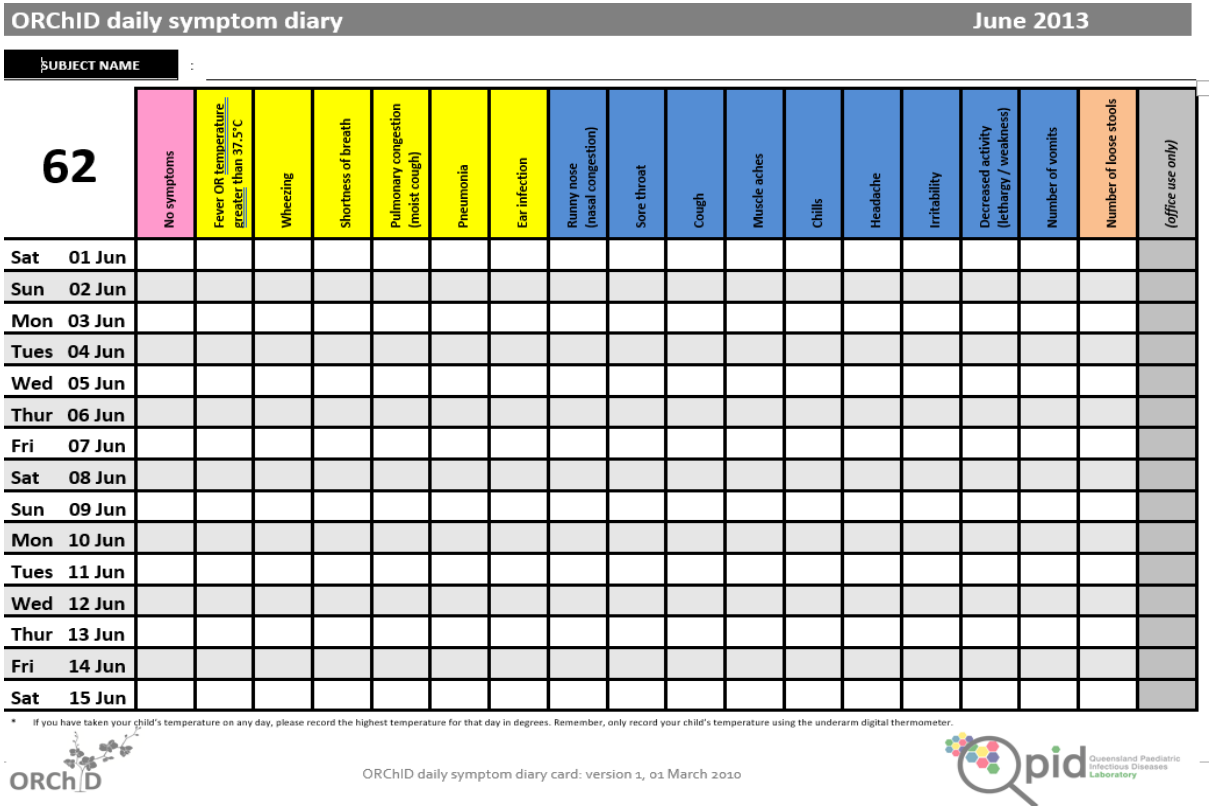
Supplementary Figure 1: Observational Research in Childhood Infectious Diseases study Symptom diary

Symptom diary for the first 2-weeks of June 2013.



ORChID daily symptom diary — **June 2013**

SUBJECT NAME : _____

62	No symptoms	Fever OR temperature greater than 37.5°C	Wheezing	Shortness of breath	Pulmonary congestion (moist cough)	Pneumonia	Ear infection	Runny nose (nasal congestion)	Sore throat	Cough	Muscle aches	Chills	Headache	Irritability	Decreased activity (lethargy / weakness)	Number of vomits	Number of loose stools	*(office use only)*
Sat 01 Jun																		
Sun 02 Jun																		
Mon 03 Jun																		
Tues 04 Jun																		
Wed 05 Jun																		
Thur 06 Jun																		
Fri 07 Jun																		
Sat 08 Jun																		
Sun 09 Jun																		
Mon 10 Jun																		
Tues 11 Jun																		
Wed 12 Jun																		
Thur 13 Jun																		
Fri 14 Jun																		
Sat 15 Jun																		

* If you have taken your child's temperature on any day, please record the highest temperature for that day in degrees. Remember, only record your child's temperature using the underarm digital thermometer.

ORChID

ORChID daily symptom diary card: version 1, 01 March 2010

Qpid Queensland Paediatric Infectious Diseases Laboratory

Supplementary Table 1: Association of RSV subtypes with respiratory illness (10,811 swabs, 82,036 person-days of observation)*

	Episodes	Asymptomatic episodes n, %	ARI n, %	URI n, %	ALRI n, %
RSV combined†	82	22 (26.8)	60 (73.2)	26 (31.7)	34 (41.5)
Shedding duration					
1 week	64	17 (26.6)	47 (73.4)	21 (32.8)	26 (40.6)
2 weeks	10	4 (40.0)	6 (60.0)	2 (20.0)	4 (40.0)
3 weeks	2	0 (0.0)	2 (100.0)	1 (50.0)	1 (50.0)
4 weeks	3	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)
5 weeks	3	1 (33.3)	2 (66.7)	2 (66.7)	0 (0.0)
RSV-A episodes†	59	18 (30.5)	41 (69.5)	16 (27.1)	25 (42.4)
Shedding duration					
1 week	44	13 (29.6)	31 (70.5)	13 (29.6)	18 (40.9)
2 weeks	9	4 (44.4)	5 (55.6)	2 (22.2)	3 (33.3)
3 weeks	1	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
4 weeks	3	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)
5 weeks	2	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)
RSV-B episodes†	24	4 (16.7)	20 (83.3)	11 (45.8)	9 (37.5)
Shedding duration					
1 week	21	4 (19.1)	17 (81.0)	9 (42.9)	8 (38.1)
2 weeks	1	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
3 weeks	1	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
4 weeks	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5 weeks	1	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)

Abbreviations: ALRI: acute lower respiratory infection; ARI: acute respiratory infection; RSV: respiratory syncytial virus; URI: upper respiratory infection.

*104 RSV-positive swabs (85 episodes) in total, of which 101 RSV-positive swabs (82 episodes) were linked to symptom diaries.

†1 swab was positive for both RSV-A and RSV-B and was a symptomatic case.

Supplementary Table 2: Sensitivity analysis for association between peak RSV virus loads (inverse cycle threshold value) and presence of symptoms for each episode in children in the Observational Research in Childhood Infectious Diseases cohort. Episodes were included if a negative swab was returned in the week preceding the incident swab (n=72 episodes from 154 children)*

	Asymptomatic Mean (SD)	Symptomatic Mean (SD) †	Mean Difference (95%CI)‡
RSV combined	33.7 (3.8) [n=17]	29.9 (3.5) [n=55]	3.7 (1.7, 5.7)
RSV-A	33.4 (4.1) [n=14]	29.7 (3.3) [n=38]	3.6 (1.4, 5.9)
RSV-B	35.2 (1.1) [n=3]	30.5 (3.8) [n=18]	4.7 (0.0, 9.4)

Abbreviations: CI: confidence interval; RSV: respiratory syncytial virus; SD: standard deviation.

72 episodes were linked to a negative swab in the preceding week and to symptom diaries.

*Episodes were included if there was a negative swab returned in the week (defined as 7 +/- 3 days) prior to the incident swab.

†1 swab was positive for both RSV-A and RSV-B and was a symptomatic case.

‡ Linear regression was used with symptomatic episodes as the referent.

Supplementary Table 3: Association between peak RSV virus loads (inverse cycle threshold value) for each episode and shedding duration in children in the Observational Research in Childhood Infectious Diseases cohort (n=85 episodes from 154 children, 10,811 swabs, 82,036 person-days of observation).*

	RSV combined (N=85)			RSV-A episodes† (N=62)			RSV-B episodes† (N=24)		
Shedding duration	Number of Episodes	Ct value Mean (SD)	Mean difference (95% CI)	Number of Episodes	Ct value Mean (SD)	Mean difference (95% CI)	Number of Episodes	Ct value Mean (SD)	Mean difference (95% CI)
1 week	67	30.8 (3.9)	Ref	47	30.7 (3.9)	Ref	21	31.0 (4.0)	Ref
2 weeks	10	30.9 (4.8)	0.2 (-2.5, 2.9)	9	31.1 (5.1)	0.4 (-2.5, 3.4)	1	29.9 (-)	-1.1 (-9.7,7.5)
3 weeks	2	29.7 (0.5)	-1.1 (-6.9, 4.7)	1	29.9 (-)	-0.7 (-8.9,7.6)	1	29.4 (-)	-1.7 (-10.3, 6.9)
4 weeks	3	31.6 (3.9)	0.8 (-4.0, 5.5)	3	31.6 (3.9)	0.9 (-3.9,5.8)	0	- (-)	-
5 weeks	3	27.4 (4.9)	-3.4 (-8.1, 1.4)	2	25.1 (3.9)	-5.6 (-11.5, 0.3)	1	32.0 (-)	1.0 (-7.6, 9.6)

Abbreviations: ALRI: acute lower respiratory infection; ARI: acute respiratory infection; Ct: cycle threshold; RSV: respiratory syncytial virus; URI: upper respiratory infection.

*104 RSV-positive swabs (85 episodes) in total. Mutually exclusive episodes.

†1 swab was positive for both RSV-A and RSV-B.

Supplementary Table 4: Children with recurrent RSV infections*

Participant identification number	Age (months) at first detection	Symptoms association with first detection	Age (months) at second detection	Symptoms at second detection
4	10	URI	17	URI
56	13	URI	22	URI
62	8	No symptoms	22	ALRI
118	10	ALRI	17	No symptoms
127	4	No symptoms	8	No symptoms
161	5	ALRI	11	No symptoms

Abbreviations: ALRI: acute lower respiratory infection; RSV: respiratory syncytial virus; URI: upper respiratory infection.

*All recurrent RSV infections were from RSV-A.

Supplementary Table 5: Co-detection of RSV with other viruses and bacteria (n=90 higher-quality swabs, 88 higher-quality swabs with symptom diary data)*

			ARI symptoms†					
			None (n=36)		URI (n=21)		ALRI (n=31)	
Co-detection	N	%	N	%	N	%	N	%
Any virus								
Any other virus detected	26	28.9	11	42.3	3	15.4	11	38.5
No virus co-detected	64	71.1	25	39.1	18	28.1	20	31.3
Human rhinovirus								
Huma rhinovirus detected	14	15.6	5	35.7	1	14.3	7	42.9
No Human rhinovirus detected	76	84.4	31	40.8	20	26.3	24	31.6
Human polyomavirus WU/KI								
Human polyomavirus detected	7	7.8	3	42.9	0	14.3	3	42.9
No Human polyomavirus detected	83	92.2	33	39.8	21	25.3	28	32.5
Human bocavirus-1								
Human bocavirus-1 detected	3	3.3	3	100.0	0	0.0	0	0.0
No Human bocavirus-1 detected	87	96.7	33	37.9	21	25.3	31	34.5
Adenovirus								
Adenovirus detected	3	3.3	0	0.0	0	0.0	3	100.0
No Adenovirus detected	87	96.7	36	41.4	21	25.3	28	31.0
Human coronavirus‡								
Human coronavirus detected	1	1.1	0	0.0	1	100.0	0	0.0
No Human coronavirus detected	89	98.9	36	41.4	20	22.5	31	34.8
Human metapneumovirus								
Human metapneumovirus detected	1	1.1	0	0.0	0	0.0	1	100.0
No Human metapneumovirus detected	89	98.9	36	40.4	21	23.6	30	33.7
Any bacteria								
Any bacteria detected	65	72.2	26	40.0	13	20.0	24	36.9
No bacteria co-detected	25	27.8	10	40.0	8	32.0	7	28.0
S. pneumoniae								
S. pneumoniae detected	48	53.3	18	37.5	9	18.8	20	97.9
No ***S. pneumoniae*** detected	42	46.7	18	42.9	12	28.6	11	97.6
M. catarrhalis								
M. catarrhalis detected	43	47.8	17	39.5	8	18.6	16	37.2
No ***M. catarrhalis*** detected	47	52.2	19	40.4	13	27.7	15	31.9
H. influenzae								
H. influenzae detected	17	18.9	8	47.1	2	11.8	5	29.4
No ***H. influenzae*** detected	73	81.1	28	38.4	19	26.0	26	35.6

Abbreviations: ALRI: acute lower respiratory infection; ARI: acute respiratory infection; RSV: respiratory syncytial virus; URI: upper respiratory infection.

**Bordetella pertussis* (n=1), *B. parapertussis* (n=4), *Chlamydia pneumoniae* (n=2), *Mycoplasma pneumoniae* (n=0), and *Simkania negevensis* (n=0) were detected rarely, or not at all, and were not included in the analysis.

†2 swabs missing symptom diary entry.

‡includes alpha-coronaviruses 229E and NL63 and lineage A beta-coronaviruses HKU1 and OC43.

Supplementary Table 6: Association between RSV detections and other respiratory virus and bacterial detections (n=8,101 higher-quality swabs)*

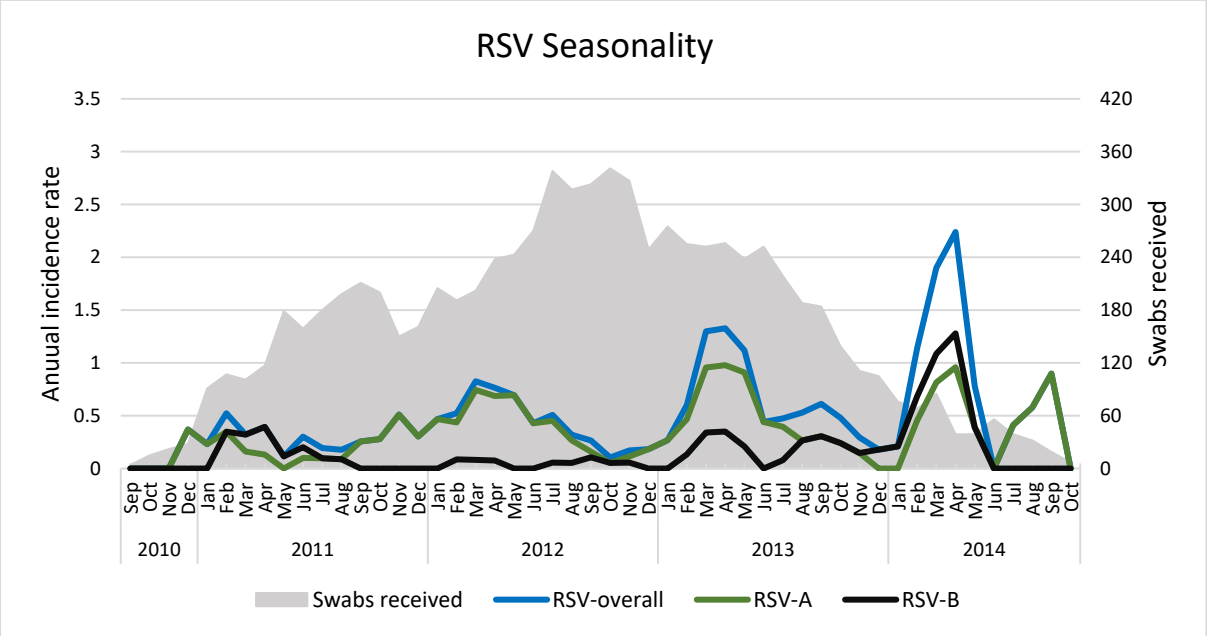
Virus	No RSV	RSV	Relative risk (95%CI)	Relative risk (95%CI)
	No. (%)	No. (%)	Crude	Adjusted†
Total swabs	8011 (98.9)	90 (1.1)		
No HRV	5960 (98.7)	76 (1.3)	1.0	1.0
HRV	2051 (99.3)	14 (0.7)	**0.54 (0.31─0.95)**	**0.38 (0.22─0.68)**
No HPyV WU/KI	7756 (98.9)	83 (1.1)	1.0	1.0
HPyV	255 (97.3)	7 (2.7)	**2.52 (1.18─5.40)**	**2.37 (1.09─5.11)**
No HBoV-1	7877 (98.9)	87 (1.1)	1.0	1.0
HBoV-1	134 (97.8)	3 (2.2)	2.00 (0.64─6.26)	1.57 (0.50─4.89)
No AdV	7910 (98.9)	87 (1.1)	1.0	1.0
AdV	101 (97.1)	3 (2.9)	2.65 (0.85─8.25)	2.09 (0.67─6.51)
No HCoVs	7859 (98.9)	89 (1.1)	1.0	1.0
HCoVs	152 (99.4)	1 (0.6)	0.58 (0.08─4.16)	0.54 (0.08─3.85)
No HMPV	7985 (98.9)	89(1.1)	1.0	1.0
HMPV	26 (96.3)	1 (3.7)	3.36 (0.49─23.3)	3.47 (0.51─23.9)
No *S. pneumoniae*	4623 (99.1)	42 (0.9)	1.0	1.0
S. pneumoniae	3388 (98.6)	48 (1.4)	**1.55 (1.03─2.34)**	1.07 (0.69─1.67)
No *M. catarrhalis*	4905 (99.1)	47 (0.9)	1.0	1.0
M. catarrhalis	3106 (98.6)	43 (1.4)	1.44 (0.95─2.17)	0.98 (0.63─1.55)
No *H. influenzae*	6833 (98.9)	73 (1.1)	1.0	1.0
H. influenzae	1178 (98.6)	17 (1.4)	1.35 (0.80─2.27)	0.94 (0.54─1.63)

Abbreviations: AdV: adenovirus; HBoV-1: human bocavirus-1; HMPV: human metapneumovirus; HPyV: human polyomaviruses; HRV: human rhinovirus; RR: relative risk; RSV: respiratory syncytial virus.

*****_Bordetella pertussis_ (n=1), _B. parapertussis_ (n=4), _Chlamydia pneumoniae_ (n=2), _Mycoplasma pneumoniae_ (n=0), and _Simkania negevensis_ (n=0) were detected rarely, or not at all, and were not included in the analysis.

†Multivariable regression adjusted for age, older child in the household, childcare attendance and season of detection.

Supplemental Figure 2. Seasonality of the total number of respiratory syncytial virus (RSV) episodes, and RSV-A, and RSV-B subtypes, plotted as the 3-month moving average of annual incidence rates. Data collected from September 2010 to October 2014.



Supplementary Table 7: Number of children, child-years, single new RSV detection episodes, and incidence rates in children in the Observational Research in Childhood Infectious Diseases cohort (n=8101 higher-quality swabs).

Risk factor	Number of children	Child-years observation	New RSV episodes *	Incidence rate per child-year (95% CI)	Incidence Rate Ratio (95% CI)	Incidence Rate Ratio (95% CI)
					Unadjusted	Adjusted†
Age (months)						
0─<3	157	22.57	2	0.09 (0.02─0.35)	**0.14 (0.04─0.61)**	0.23 (0.05─1.05)
3─<6	144	21.74	7	0.32 (0.15─0.68)	0.54 (0.24─1.19)	0.76 (0.30─1.90)
6─<12	136	41.21	21	0.51 (0.33─0.78)	0.85 (0.50─1.43)	1.07 (0.61─1.89)
12─<24	120	69.83	42	0.60 (0.44─0.81)	Reference	Reference
Sex						
Male	75	69.27	32	0.46 (0.33─0.65)	Reference	Reference
Female	82	86.32	40	0.46 (0.34─0.63)	1.00 (0.63─1.60)	0.96 (0.60─1.53)
Season of birth						
Summer (December-February)	42	45.28	20	0.44 (0.28─0.68)	Reference	Reference
Autumn (March-May)	30	29.46	13	0.44 (0.26─0.76)	1.00 (0.50─2.00)	1.21 (0.59─2.48)
Winter (June-August)	43	39.16	21	0.54 (0.35─0.82)	1.21 (0.66─2.24)	1.10 (0.57─2.11)
Spring (September-November)	42	41.46	18	0.43 (0.27─0.69)	0.98 (0.52─1.86)	0.92 (0.48─1.76)
Type of delivery						
Vaginal	107	103.54	53	0.51 (0.39─0.67)	Reference	Reference
Caesarean	50	51.82	19	0.37 (0.23─0.57)	0.72 (0.42─1.21)	0.73 (0.43─1.25)
Gestational age at birth						

39-41 weeks	122	123.07	57	0.46 (0.36─0.60)	Reference	Reference
36-38 weeks	35	32.30	15	0.46 (0.28─0.77)	1.00 (0.57─1.77)	1.15 (0.65─2.03)
Season of acquisition						
Summer (December-February)	145	34.73	10	0.29 (0.15─0.54)	Reference	Reference
Autumn (March-May)	146	38.15	37	0.97 (0.70─1.33)	**3.37 (1.68─6.77)**	**3.22 (1.60─6.48)**
Winter (June-August)	141	43.11	18	0.42 (0.26─0.66)	1.45 (0.67─3.14)	1.33 (0.61─2.89)
Spring (September-November)	141	39.37	7	0.18 (0.08─0.37)	0.62 (0.24─1.62)	0.59 (0.22─1.55)
Family history						
Neither parent has asthma/eczema	79	71.65	38	0.53 (0.39─0.73)	Reference	Reference
Either parent has asthma/eczema	78	83.71	34	0.41 (0.29─0.57)	0.76 (0.48─1.22)	0.74 (0.47─1.18)
Tobacco smoke exposure (n=155)						
No exposure	136	137.78	60	0.44 (0.34─0.56)	Reference	Reference
Other householder smokes	19	16.30	11	0.67 (0.37─1.22)	1.55 (0.81─2.95)	1.25 (0.65─2.41)
Household size at birth						
No older children in household	102	101.36	43	0.42 (0.31─0.57)	Reference	Reference
More than one child in household	55	54.00	29	0.54 (0.37─0.77)	1.26 (0.79─2.03)	1.44 (0.89─2.31)
Maternal education status (n=156)						
High school	20	17.55	10	0.57 (0.31─1.06)	1.20 (0.61─2.36)	1.29 (0.64─2.60)
Diploma/Certificate	37	34.90	17	0.49 (0.30─0.78)	1.08 (0.63─1.86)	1.09 (0.62─1.91)
University/higher university degree	99	102.76	45	0.44 (0.33─0.59)	Reference	Reference

Mode of feeding (n=153)						
Exclusive BF beyond age 4-months	86	85.61	40	0.47 (0.34─0.64)	Reference	Reference
Non-exclusive BF by age ≤4-months	67	69.58	32	0.46 (0.33─0.65)	0.98 (0.62─1.57)	0.96 (0.60─1.54)
Childcare attendance‡						
No childcare	156	78.78	23	0.29 (0.19─0.44)	Reference	Reference
Informal childcare only	42	19.14	5	0.26 (0.11─0.63)	0.89 (0.34─2.35)	0.70 (0.26─1.91)
Formal and/or informal childcare	89	57.44	44	0.77 (0.57─1.03)	**2.62 (1.58─4.34)**	**2.00 (1.08─3.71)**

Abbreviations: BF: breast feeding; CI: confidence interval; RSV: respiratory syncytial virus.

*There were 85 incident RSV cases; 13 cases were removed due to lower-quality swabs.

†Multivariable regression adjusted for age, older children in household at birth, childcare attendance and season of acquisition.

‡Formal childcare was defined as outside homecare from a regulated childcare service, while informal care comprised non-regulated care by relatives, friends or neighbours.